QUARTERLY PROGRESS REPORT

March 1 – May 31, 2012

PROJECT TITLE: Bioremediation of Landfill Leachate and Co-Production of Biodiesel

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PROJECT WEB ADDRESS: http://biogas.ifas.ufl.edu/leachate/

Background:

A sustainable solution for dealing with landfill leachate has yet to be devised and implemented, although landfills continue to be used as a primary means of waste disposal. The long-term management of landfill leachate remains a major concern in the assurance of environmental quality, even after the closure of a landfill site. All current methods of leachate treatment are energy and cost intensive. The most common method of leachate treatment involves the transportation of leachate to off-site water treatment facilities; incurring transportation and tipping fees, while consuming fossil resources and emitting CO_2. Algal bioremediation may provide a means for effective on-site leachate treatment with major benefits over current methods. These benefits include the reduction of environmentally noxious compounds (e.g. ammonia), oxidation of organic compounds, and the co-production of biodiesel.

The purpose of this research project is to identify algae that can effectively remediate landfill leachate. The study will characterize native Floridian algae for their tolerance of landfill leachate, effectiveness at remediating the landfill leachate, and the potential of the algal biomass as a feedstock for biodiesel production. The research conducted under this project will lay the biological foundation for implementing algal bioremediation of landfill leachate in Florida.

Work Accomplished During this Reporting Period:

Summary

Results obtained in the previous quarter showed that pH control using CO_2 as an acidulant increased the tolerance of *Chlorella cf. ellipsoidea* to landfill leachate. However, toxicity is largely dependent on the organism tested (Ward *et al.* 2002). Therefore, empirical testing was continued with a different, unrelated organism to confirm previous findings and the potential universality of results. Toxicity experiments were conducted with the Floridian alga, *Scenedesmus* sp., under identical conditions to those used with *Chlorella cf. ellipsoidea* in the previous quarter. *Scenedesmus* sp. exhibited similar results; it was found to be insensitive to sodium, chloride, and ammonium ion, but proved sensitive to unionized ammonia. Furthermore, when the pH was controlled via CO_2, *Scenedesmus* sp. was found to exhibit moderate growth in 100% landfill leachate, potentially eliminating the need for dilution of landfill leachate with groundwater prior to algal bioremediation.

Objective 1: *Characterize algae tolerant to landfill leachate.*

Methods of toxicity testing

All algal growth tests in this quarter were conducted in 250 mL Erlenmeyer flasks under light levels of 150 $\mu E/m^2/s$, conditions identical to those applied in the previous quarter. Tests were carried out either on orbital shakers operated at 140 rpm or in the case of pH control by CO_2, under aeration. Algal growth was measured by chlorophyll fluorescence (Relative Fluorescent Units at 680 nm). All experiments were executed in triplicate and were paired with leachate controls under identical conditions. This quarterly report describes the characterization of the isolated alga *Scenedesmus* sp. (ISO2).

Sodium chloride toxicity test

As noted in the previous quarter, the sodium and chloride contents of Alachua County Southwest landfill leachate (LL) are relatively high (2,713 mg/L and 1,933 mg/L, respectively) compared to standard freshwater algae culture media (81.6 mg/L and 126 mg/L, respectively, for Bold's Basal Medium). High sodium and chloride were thus identified as potential factors in the toxicity exhibited by cultures grown in high concentrations (>10%) of LL.

Testing was undertaken with analytical grade sodium chloride dissolved in deionized water at concentrations in excess of those found in the LL. These tests elucidated the effect of sodium chloride on the alga under laboratory cultivation conditions. Toxicity tests on *Scenedesmus* sp. isolated from the ACSW Landfill showed no toxicity symptoms due to NaCl levels of 5.84 g/L, 125% those found in LL (~4.65 g/L) (Figure 1). Sodium and chloride at levels found in the LL are therefore disregarded as primary toxicants to *Scenedesmus* sp., which agrees with previous findings for *Chlorella cf. ellipsoidea*. Although neither organism showed a toxicity response, *Scenedesmus* appears to have a greater tolerance to sodium chloride than *Chlorella cf. ellipsoidea*.

Ammonium chloride toxicity tests

Experimental tests were undertaken to examine the toxicity of nitrogen in the ammoniacal form, at concentrations comparable with LL, on *Scenedesmus* sp. As chloride had already been disproved as a toxicity factor for the experimental culture, the pure salt ammonium chloride was used as a testing reagent for determining the impact of ammoniacal nitrogen. Tests under laboratory conditions showed no toxic effects on *Scenedesmus* sp. at 1000 mg/L of N, a concentration slightly in excess of ACSW landfill leachate. In fact, growth was observed under these high ammonium conditions (Figure 2).

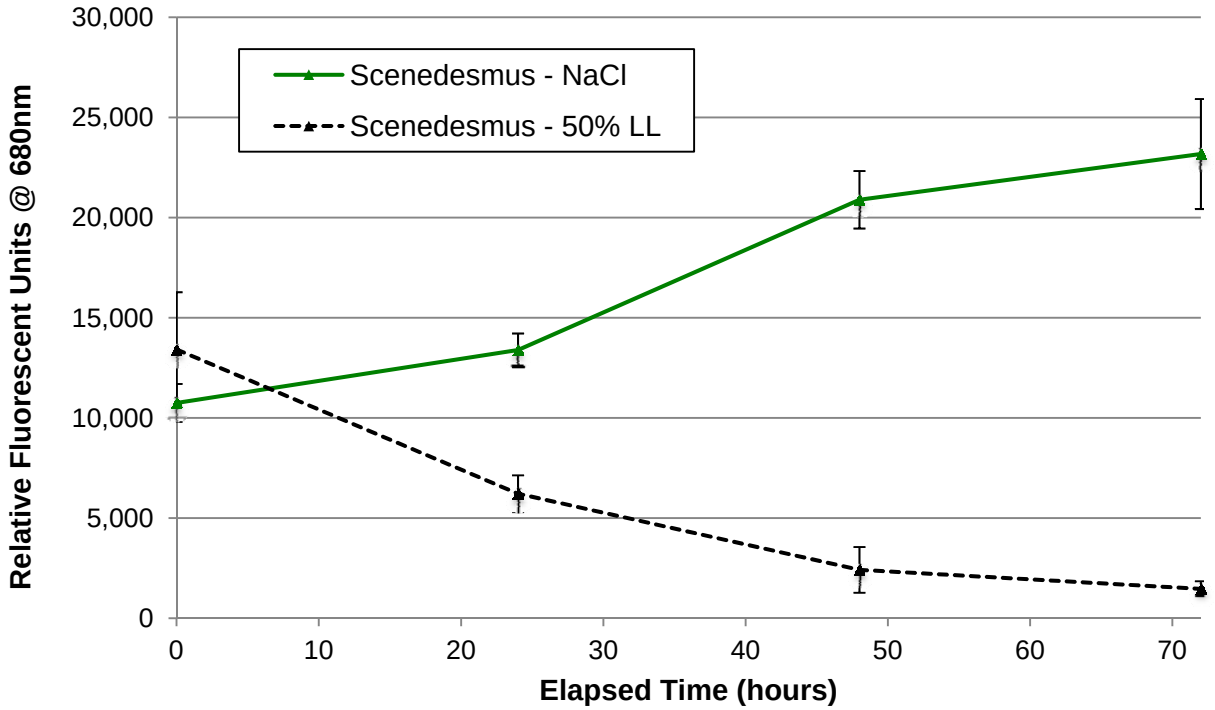

Figure 1. Effects of Sodium Chloride (5.84 g/L) on growth of *Scenedesmus* sp.compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

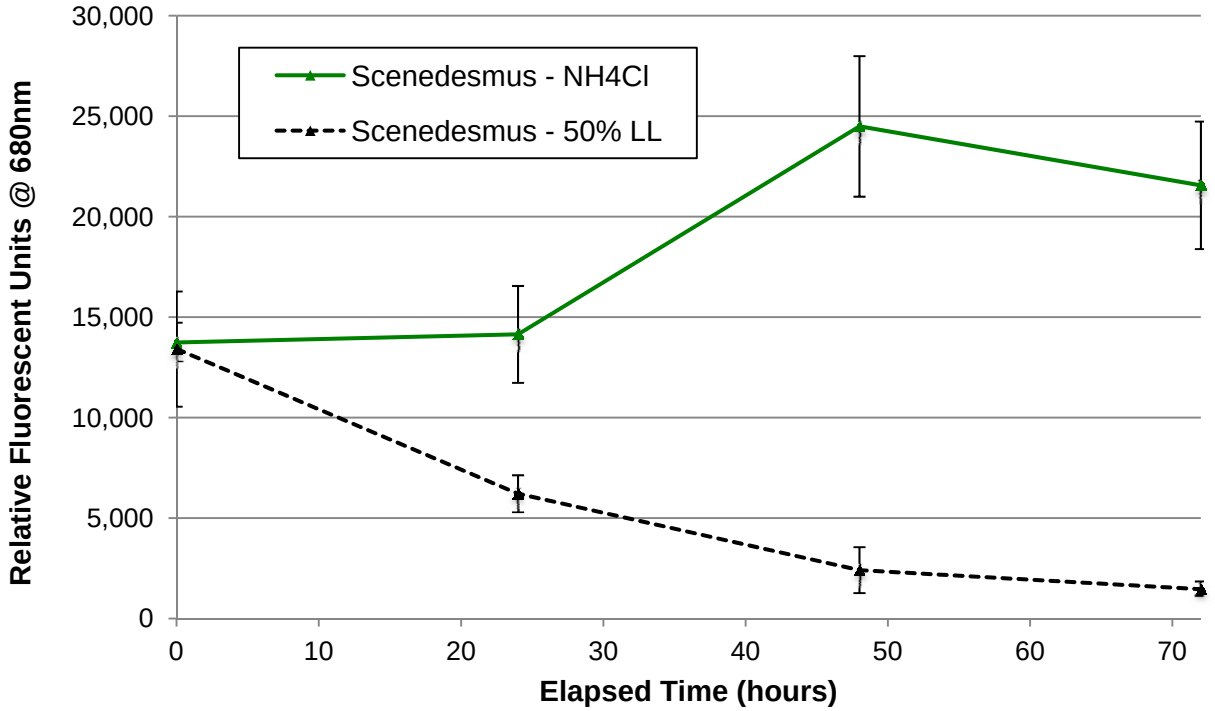

Figure 2. Effects of Ammonium Chloride (3.189 g/L), at pH 7, on growth of *Scenedesmus* sp. compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

Effect of pH on the toxicity of ammonium chloride

The pH levels of the cultures in the NH_4Cl medium remained nearly neutral during the experiment. Under neutral pH conditions, the majority of total ammoniacal nitrogen (TAN) is in the ionized form, which is less toxic (Kallqvist and Svenson 2003). Thus, the experiment only tested the toxicity of the charged ammonium ion (NH_4^+), which was not toxic to the experimental organism even at high concentrations equivalent to those found in 100% LL. Therefore, the *Scenedesmus* sp. was subjected to the same experimental concentration of TAN (1000 mg/L-N); except the pH of the medium was experimentally elevated to the pKa (ionization constant) of ammonium/ammonia (9.26 @ 25 °C). At the pKa value, the ionized and unionized forms of ammoniacal nitrogen are present in equal fractions.

When pH was artificially elevated, *Scenedesmus* sp. exhibited immediate toxic effects, similar to the alga tested in the previous quarter (Figure 3). These effects closely resembled the toxic impacts of landfill leachate on this organism which were previously observed. Furthermore, the artificially induced pH of 9.26 closely resembled the pH of LL after aeration in previous experiments. This experiment confirmed that *Scenedesmus* sp. is also sensitive to unionized ammonia.

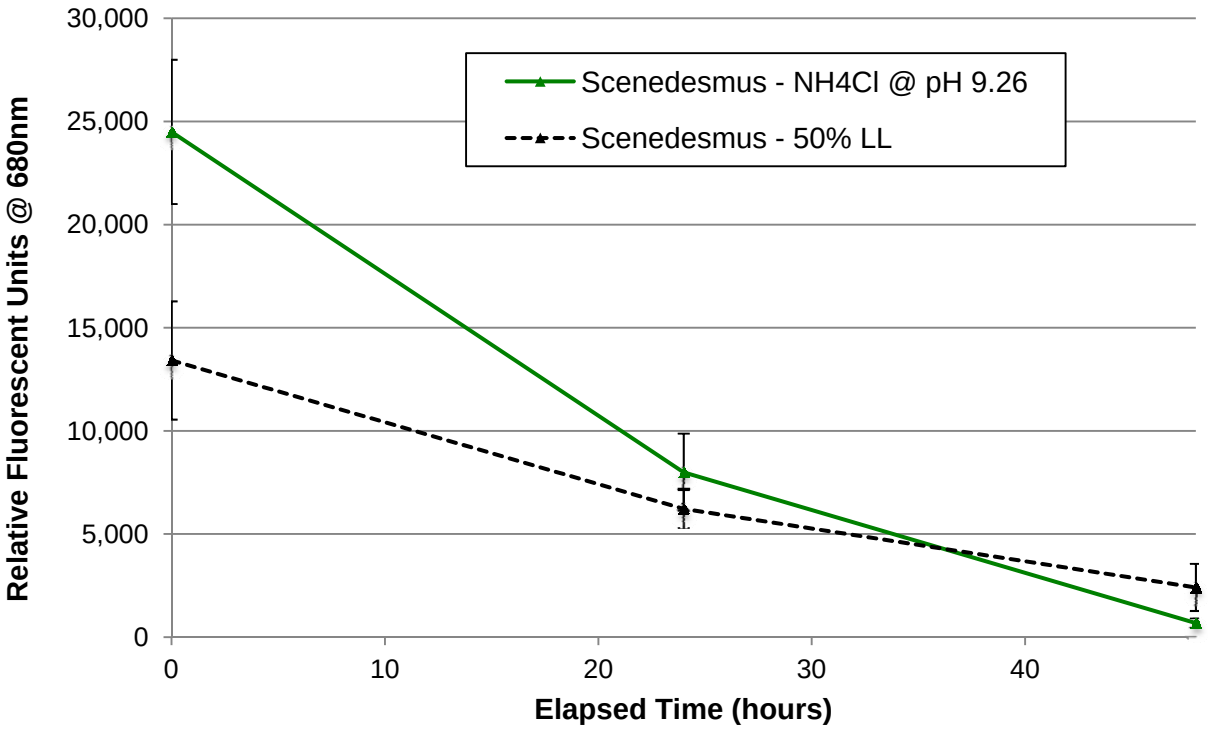

Figure 3. Effects of Ammonium Chloride (3.189 g/L), at pH 9.26, on growth of *Scenedesmus* sp. compared with 50% landfill leachate (error bars represent standard deviation of triplicate cultures).

Effect of pH control on algae cultivation in landfill leachate

Free ammonia appears to be the primary toxicant to *Scenedesmus* sp. as well as to *Chlorella cf. ellipsoidea*. pH control should allow the ammoniacal nitrogen within the leachate to remain ionized (NH_4^+) and therefore nontoxic. Regulation of pH should also allow the cultivation of *Scenedesmus* sp. in higher concentrations of landfill leachate. *Scenedesmus* sp. was grown under CO_2 –regulated pH conditions in 50% and 100% landfill leachate. Experimental treatments were potentiometrically regulated between pH 6.8 and pH 7.2 by the addition of gaseous CO_2, which dissociated in water to form carbonic acid (H_2CO_3). The pH levels of control cultures were not regulated. *Scenedesmus* sp. showed adaptation and growth in both 50% and 100% landfill leachate under pH regulation with CO_2 (Figure 4 and 5). The maximum average fluorescence observed for this species in 50% LL was 71,125 RFU, over 3-times the maximum observed in *Chlorella cf. ellipsoidea* (22,596 RFU). Maximum average fluorescence for *Scenedesmus* in 100% LL was 13,453 RFU. All control cultures without pH regulation did poorly, exhibiting the previously observed toxicity effects of LL.

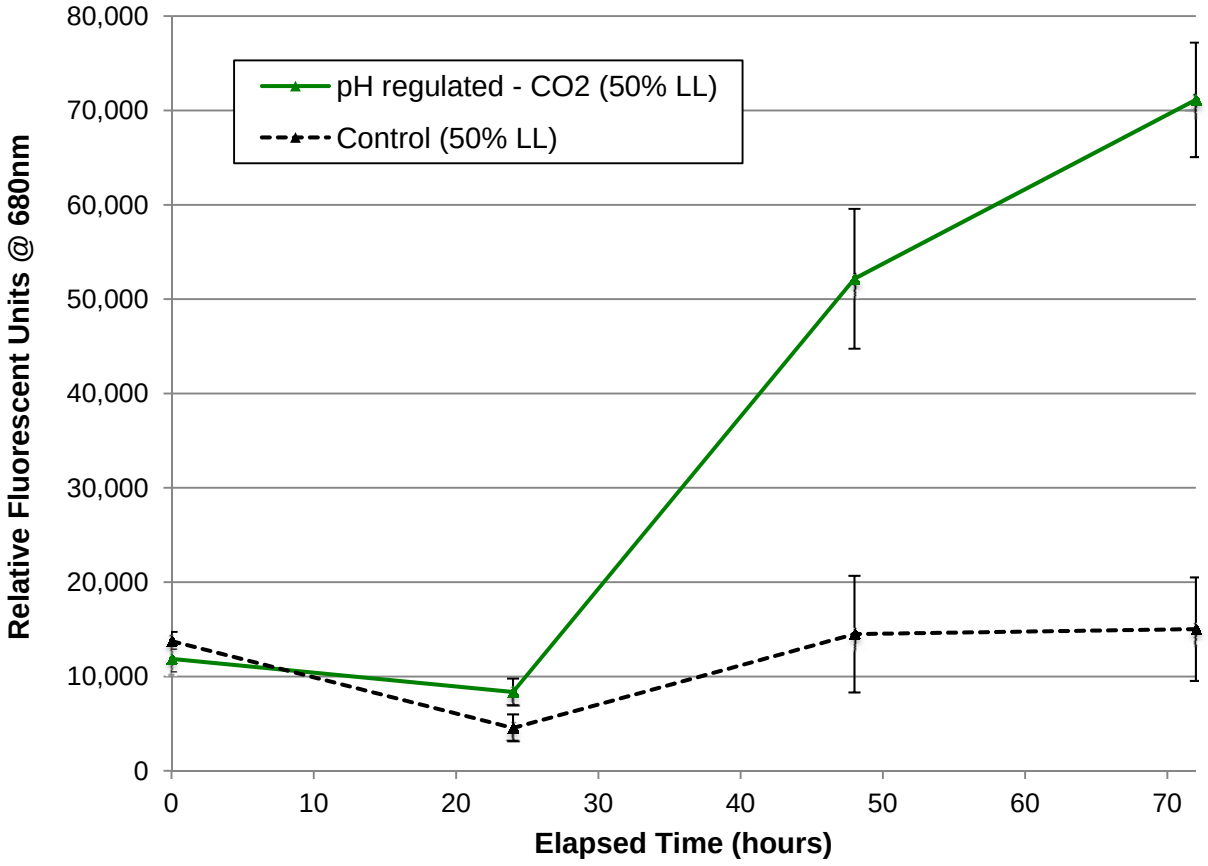

Figure 4. Effect of pH regulation via CO_2 on the cultivation of *Scenedesmus* sp. in 50% landfill leachate compared to growth in 50% landfill leachate without pH regulation (error bars represent standard deviation of triplicate cultures).

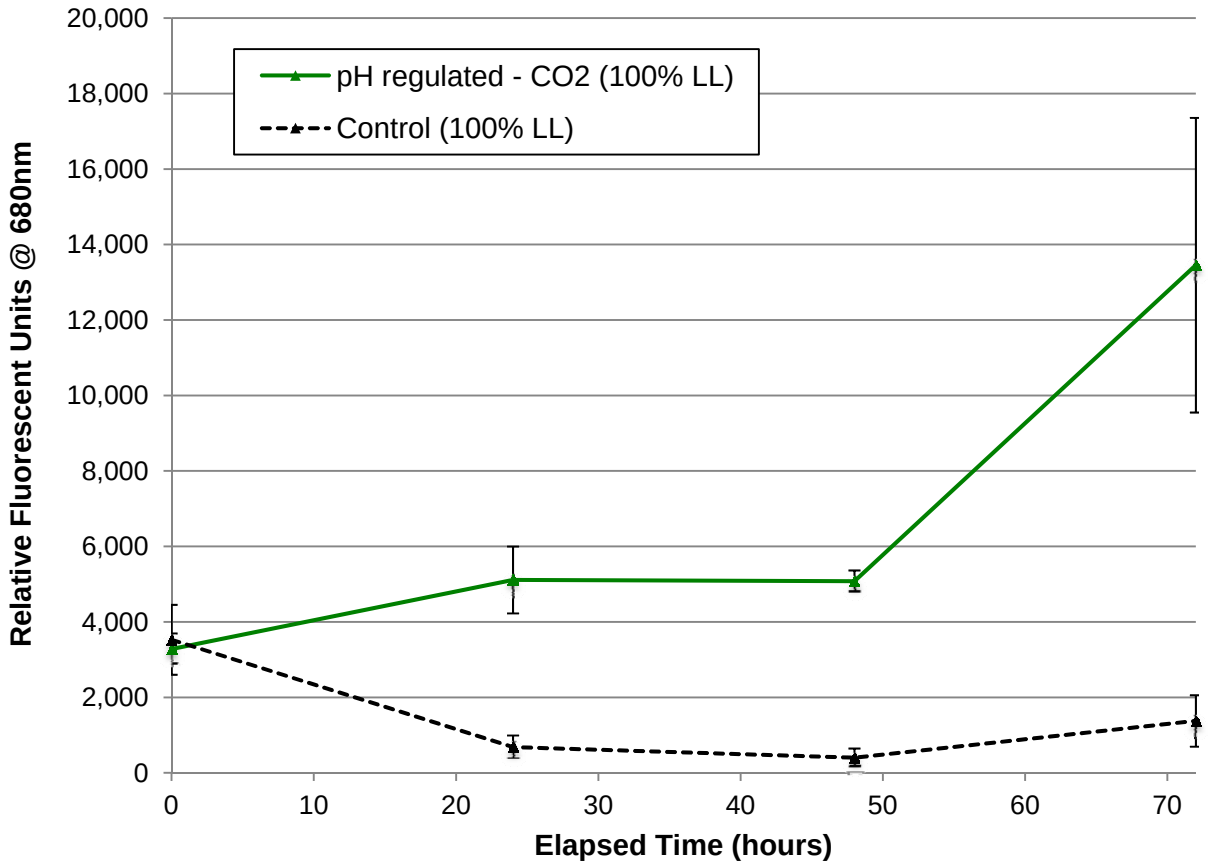

Figure 5. Effect of pH regulation via CO_2 on the cultivation of *Scenedesmus* sp. in 100% landfill leachate compared to growth in 100% landfill leachate without pH regulation (error bars represent standard deviation of triplicate cultures).

Objective 2: *Determine leachate bioremediation potential of algae*.

Scenedesmus sp. was cultivated in 500 mL flasks to test ammonia remediation capacity at 50% landfill leachate. The pH levels of the *Scenedesmus* culture and a landfill leachate control were regulated via CO_2. Preliminary results showed that the alga culture reduced total ammonia nitrogen within the landfill leachate by 78%, compared to a 10% reduction in the control (Figure 6).

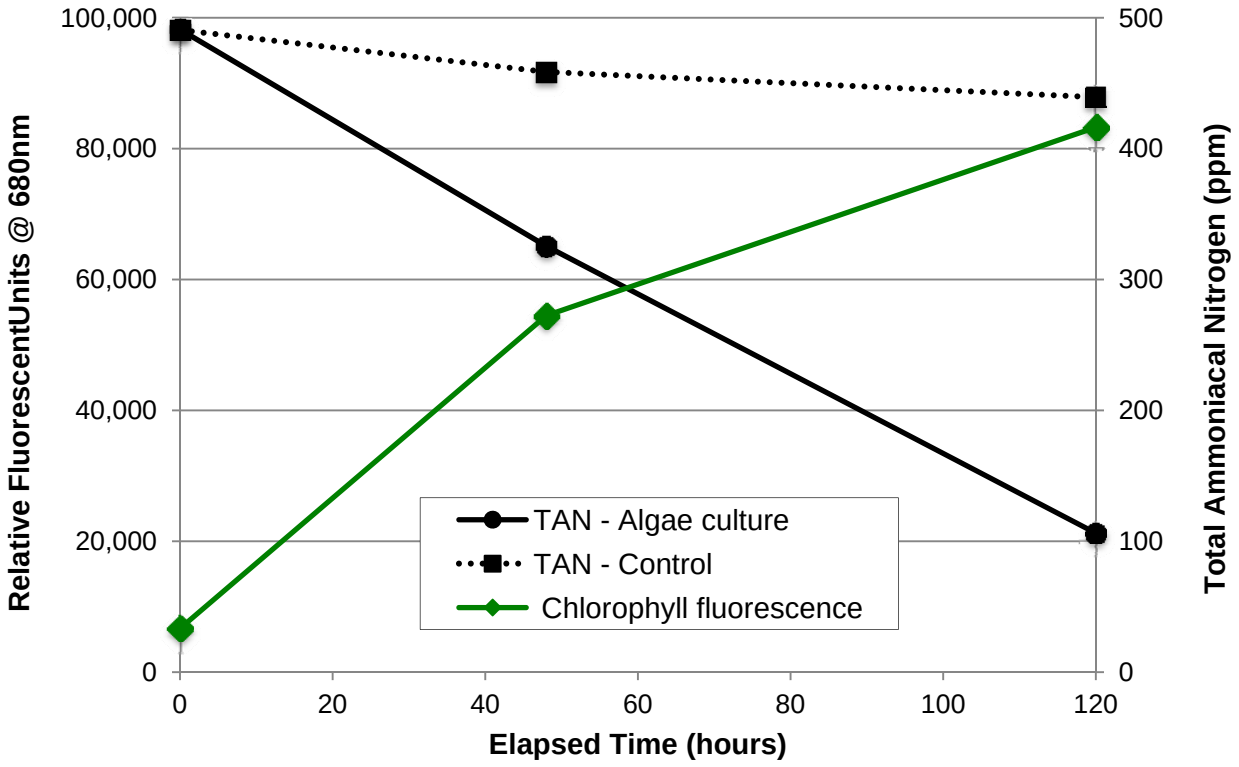

Figure 6. Remediation of total ammonia nitrogen (TAN) within 50% landfill leachate by the native Floridian alga, *Scenedesmus* sp. pH regulated via CO_2 in both the *Scenedesmus sp.* culture (solid black line: ●) and the landfill leachate control (dashed line: ■). Algal growth is shown as an increase in chlorophyll fluorescence (green line: ◆).

Objective 3: *Examine the viability of biodiesel production from algal biomass.*

Although *Scenedesmus* sp. tolerates a higher concentration of landfill leachate, *Chlorella cf. ellipsoidea* appears to store more oils and thus is more suited for biodiesel production. *Scenedesmus* sp., if unsuitable for biodiesel production, may be suitable for biogas production via anaerobic digestion of the algal biomass. The advantage of growing at a high concentration of landfill leachate may outweigh the production of biodiesel, but necessitates further testing.

Conclusions

Toxicity tests conducted in this quarter with *Scenedesmus* sp. confirm the results discovered in previous experiments with *Chlorella cf. ellipsoidea*, which delineated unionized ammonia as the primary toxicant in landfill leachate. *Scenedesmus* sp. shows excellent growth in 50% LL as well as moderate growth in 100% LL, potentially eliminating the need for diluting landfill leachate. Based on these tests, *Scenedesmus* appears to be a superior choice for cultivation on landfill leachate, due to its tolerance of undiluted landfill leachate. Further optimization of growth in landfill leachate seems feasible given the remarkable increase in maximum percentage

LL tolerated by these two organisms with only the modulation of a single parameter and without long-term selection or breeding. Cultivation results may be improved by several means, including: adaptation of the cultures to landfill leachate by successive generations of growth within landfill leachate (artificial selection), or by further optimizing the culture environment (light, CO_2 diffusion mechanisms, mixing regime, trace nutrients, *etc.*). As noted previously, pH regulation via CO_2 in undiluted landfill leachate was problematic due to the high concentration of bicarbonate within the landfill leachate and the aeration-based mixing of the culture. Using mineral acids to neutralize the pH and/or CO_2 to maintain the pH may be an appropriate strategy for future cultivation experiments.

Laboratory cultivation methods in which *Scenedesmus* sp., a Floridian algal isolate, grew in undiluted raw landfill leachate were developed through empirical testing. Methods for cultivating algae within undiluted landfill leachate have again progressed beyond concentrations reported within the literature and may increase the feasibility of algae-based landfill leachate bioremediation. The progress made towards establishing the fundamental requirements for supporting algal growth in high concentrations of landfill leachate undergirds future research objectives of evaluating the remediation effectiveness and biofuel production potential of this innovative and environmentally sound strategy for landfill leachate management.

References:

Kallqvist. T. and Svenson, A. (2003). Assessment of ammonia toxicity in tests with the microalga, *Nephroselmis pyriformis*, Chlorophyta. *Water Research* **37**(3):477-484.

Ward, M.L., Bitton, G., Townsend, T. and Booth, M, (2002). Determining toxicity of leachates from Florida municipal solid waste landfills using a battery-of-tests approach. *Environmental Toxicology* **17**(3):258-266.

Information Dissemination Activities:

- We have made updates and additions to the project website, including details of events, news, photos, presentations, and posters. The website is available at: http://biogas.ifas.ufl.edu/leachate/

- On March 13th and 15th, 2012, the BioEnergy and Sustainable Technology (BEST) Laboratory held tours for UF undergraduate students in the Environmental Science and Humanities Lab (EES 3000L). Students learned about landfill leachate and our research on the use of algae to bioremediate landfill leachate. Fifteen students toured the labs on each of these days.

- We hosted the Academy of Environmental Sciences (AES) from Crystal River, Citrus County, at the BEST Lab on April 17th, 2012. Students from the AES magnet middle school learned about the importance of environmental quality and bioremediation, and specifically

about landfill leachate and the need to remediate before discharging into the natural environment. Twenty-five students attended.

- On April 30th 2012, Scott Edmundson and Reginald Toussaint presented BEST Lab research at the Sweetwater Branch Academy Elementary School, Gainesville, FL. Four presentations were made to Kindergarten, 1st and 2nd, 3rd, and 4th and 5th grade students, respectively. Altogether, 165 students learned about algal photosynthesis, waste management, and biological remediation. Students enjoyed watching an active photobioreactor growing algae as well as receiving an algae coloring sheet and BEST Lab stickers. A question and answer session at the end of each presentation engaged the students in a discussion about sustainability and waste management. A follow up trip has been requested by the students' teachers, recommending that students participate in hands-on experiments.

- On Thursday May 17th 2012, Tayo Olayiwola, an environmental specialist from FDEP, visited the BEST lab, touring our research projects and learning about the applications of algae bioremediation for leachate treatment and additionally about anaerobic digestion of wastes.